

Human Immunodeficiency Virus Type 1 DNA Synthesis, Integration, and Efficient Viral Replication in Growth-Arrested T Cells

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Human immunodeficiency virus type 1 (HIV-1) replicates efficiently in nonproliferating monocytes and macrophages but not in resting primary T lymphocytes. To determine the contribution of cell division to the HIV-1 replicative cycle in T cells, we evaluated HIV-1 expression, integration of proviral DNA, and production of infectious progeny virus in C8166 T-lymphoid cells blocked in cell division by treatment with either mitomycin, a DNA cross-linker, or aphidicolin, a DNA polymerase α inhibitor. The arrest of cell division was confirmed by assay of [3 H]thymidine uptake; the nondividing cells remained viable for at least 3 days after treatment. HIV-1 was expressed and replicated equally well in nondividing and dividing C8166 cells, as judged by the comparison of the levels of p24 core antigens in culture supernatants, the proportion of cells expressing HIV-1 specific antigens, the pattern and quantity of HIV-1 DNA present in the extrachromosomal and total cellular DNA fractions, and the biological activity of progeny viruses. A polymerase chain reaction-based viral DNA integration assay indicated that HIV-1 provirus was integrated in C8166 cells treated with either of the two inhibitors of cell division. Similar results were obtained by using growth-arrested Jurkat T-lymphoid cells. We conclude that cell division and cellular DNA synthesis are not required for efficient HIV-1 expression in T cells.

The proliferative status of the host cell determines the progress of the oncogenic retrovirus life cycle (5, 12, 22, 25, 38, 62). Rous sarcoma virus, spleen necrosis virus, and murine leukemia virus abort replication during reverse transcription in nondividing cells, although viral DNA synthesis resumes when the cells are stimulated to divide (5, 22, 25). Cell division has been considered to be requisite for the synthesis of full-length retroviral DNA and its integration, and so has been considered to be requisite for viral replication (5, 12, 22, 25, 38, 62).

Lentiviruses, a subfamily of *Retroviridae* (9, 42), differ fundamentally from oncogenic retroviruses in that they are relatively independent of cell division for completion of their replicative cycle. Visna virus, equine infectious anemia virus, and caprine arthritis-encephalitis virus all productively infect their natural host cells, monocytes and macrophages, in vivo and in vitro (37, 42, 70); these cells are capable of further differentiation but generally do not proliferate (14). Cell activation and/or differentiation, rather than cell division, are required for optimal replication of these viruses (20, 42). As a further distinction from oncogenic retroviruses, visna virus DNA was shown to integrate into the host cell genome during productive infection of stationary choroid ganglion cells (7, 21).

Human immunodeficiency virus type 1 (HIV-1) is a lentivirus (9, 17), and like other viruses of this subfamily, it productively infects monocytes and macrophages (13, 15, 24, 43), including end-stage mononuclear phagocytes (46, 50). Likewise, as shown with irradiated blood monocytes, cell division per se is not required for productive HIV-1 infection and viral DNA integration (65), although cell activation and/or differentiation increase virus production (15,

31, 39, 45, 61), and in proliferating monocytes virus replication may depend upon cellular DNA synthesis (33, 52). In contrast to other animal lentiviruses (18, 42), the predominant target for HIV-1 in the blood is a CD4-bearing T lymphocyte (30, 36, 51, 68). T cells divide when activated (27), and this activation of T cells is required for high-level HIV-1 production (30, 36, 68). The specific restrictions on HIV-1 replication imposed by resting T lymphocytes remain undefined, and the results of studies on HIV-1 infection in these cells are conflicting (19, 59, 68, 69). HIV-1-cell fusion (19), completion of reverse transcription (68), and HIV-1 DNA integration (59) have each been identified as the pivotal event in which HIV-1 replication arrests in resting T cells. In contrast, a study by McDougal and colleagues suggested that activation rather than proliferation of T lymphocytes determines the outcome of HIV-1 infection in these cells (36).

To investigate the contribution of the cell cycle to HIV-1 replication in T cells, we attempted to separate cell division and cellular DNA synthesis from other cellular processes required for the expression and replication of HIV-1. Because resting T lymphocytes cannot be activated without inducing cell division, we adopted an alternative approach: arresting cell division in T cells which are already susceptible to HIV-1 infection. We used two inhibitors of cellular DNA synthesis: mitomycin, an aziridine antibiotic which binds covalently to DNA and irreversibly cross-links the two strands of DNA, blocking progress of DNA polymerases (53, 67), and aphidicolin, a DNA polymerase α inhibitor (67). We show that growth-arrested T cells resemble their dividing counterparts in the proportion of cells susceptible to HIV-1 infection, levels of HIV-1 production, HIV-1 DNA pattern and integration, and infectivity of progeny virus. Thus, other mechanisms, rather than the lack of cell division, are responsible for inefficient HIV-1 replication in quiescent T lymphocytes.

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MATERIALS AND METHODS

Cell lines and viruses. CD4⁺ T-cell lines used in these studies were obtained as follows: CEM cells (11) were obtained from the American Type Culture Collection (Rockville, Md.); CR10, the HIV-1 lysis-resistant subclone of CEM, was established in our laboratory (4); the human T-cell lymphotropic virus type I (HTLV-I)-carrying C8166 cells (49) and HIV-1 chronically infected ACH-2 cells (8) were obtained from the AIDS Research and Reference Reagent repository (Rockville, Md.); Jurkat cells (66) were obtained from W. Green; and the HTLV-IIIB-infected and uninfected H9 cells (44) were obtained from R. C. Gallo. Cells were maintained in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 5% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). The HIV-1/N1T isolate (3) was propagated in CEM or CR10 cells; the HIV-1/HTLV-IIIB isolate was propagated in chronically infected H9 cells. Viruses were isolated and titered for biological activity by end-point dilution (N1T in CEM cells and HTLV-IIIB in H9 cells), as described previously (4, 63). A multiplicity of infection of 1 was defined to be the smallest amount of virus required to result in greater than 50% immunofluorescence (IF)-positive cells 3 days after infection; this is approximately 1 pg of HIV-1 p24 per cell, depending on virus preparation (63).

Arrest of cell division and HIV-1 infection of nondividing cells. C8166 cells were incubated at 37°C at 10⁶ cells per ml in the presence of 0.5 to 2.0 µg of mitomycin per ml or 0.125 to 0.5 µg of aphidicolin per ml (both from Sigma, St. Louis, Mo.). After 1 h, mitomycin was removed by extensive washing; aphidicolin was present throughout preculture and infection. Jurkat cells were treated similarly except that 0.25 µg of drugs per ml was used. All cells were incubated overnight prior to infection to ascertain a uniform arrest of cell division at the time of infection. The arrest of cell growth and cell viability were monitored, respectively, by assay of [³H]thymidine uptake (10) and trypan blue exclusion and, in some experiments, by the tetrazolium salt (MTT) assay (40). For HIV-1 infection, cells were washed, resuspended at 1 × 10⁶ cells per ml, and incubated with virus at 1 pg of viral p24 per cell (multiplicity of infection of 1). After 1 h at 37°C, excess virus was removed by washing and cells were cultured under standard conditions.

Assays of HIV-1 antigen expression. HIV-1 p24 core protein in culture supernatants or in cell lysates was measured by the enzyme-linked immunosorbent assay (Coulter Immunology, Hialeah, Fla.), following the manufacturer's instructions. A manufacturer-provided lysis buffer (which includes detergent) was used to lyse cells and to dilute samples as appropriate. A standard curve was constructed by using standards supplied in the kit and by extrapolating from the optical density to determine the amount of p24 in unknown samples. Fixed-cell IF assays for the cell-associated HIV-1 antigen expression were carried out as previously described (4).

Hirt extraction and analysis of extrachromosomal HIV-1 DNA. Cells were harvested at the indicated time points, and extrachromosomal DNA was extracted by the Hirt procedure (23) as modified by Chinsky and Soeiro (6). Total DNA recovered from 4 × 10⁶ to 5 × 10⁶ cells was loaded onto a 0.9% agarose gel for electrophoresis (the same cell number was assayed in all samples at a particular time point). After electrophoresis, the DNA was transferred to a Nytran nylon membrane (Schleicher & Schuell, Keene, N.H.) and was

analyzed for HIV-1 specific sequences by hybridization with the [³²P]dCTP-labeled 8.9-kb *SacI-SacI* fragment derived from the HIV-1/N1G-G clone (64) as probe. Autoradiography was performed over 1 to 5 days with Kodak Blue-brand film and intensifying screens.

Southern blot analysis of HIV-1 DNA. Total cellular DNA was isolated from cells 36 to 48 h after infection by detergent lysis and proteinase K digestion followed by phenol-chloroform extraction, ethanol precipitation, and spectroscopic quantitation of the DNA recovered (1); 10 to 20 µg of DNA samples was digested to completion with either *HindIII* or *SacI*, subjected to electrophoresis through a 1% agarose gel, blotted onto a Nytran nylon membrane (Schleicher & Schuell), and analyzed for HIV-1 specific sequences by hybridization. As the HIV-1 specific probe, we used a 1:1 (wt/wt) mixture of two [³²P]dCTP-labeled HIV-1 DNA-containing fragments: the 8.9-kbp *SacI-SacI* fragment derived from the N1G-G clone (64) and the 5.2-kbp pEG602 plasmid containing a 719-bp fragment of HIV-1/N1T-E long terminal repeat (LTR) (16). The hybridized filter was washed three times for 30 min at 50°C in 1× SSC (0.15 M NaCl and 0.015 M sodium citrate) supplemented with 1% sodium dodecyl sulfate before autoradiography.

A PCR-based HIV-1 DNA integration assay. The detailed procedure for detection of integrated HIV-1 DNA will be published elsewhere (54a). Briefly, high-molecular-weight cellular DNA was isolated from assayed cells by the proteinase K-phenol-chloroform extraction method (1), and 2 µg of each DNA preparation was subjected to a double digestion with *SsrI* and *XbaI* overnight at 37°C. Digested DNA was electrophoresed in a 1% low-melting-point (LMP) agarose gel; sections of the gel containing DNA fragments between 1 and 5 kbp in size were excised. DNA was extracted with the Rapid GeneClean kit (Bio 101, Inc., La Jolla, Calif.) and was subjected to ligation in the presence of 33.3 Weiss units of T4 DNA ligase (New England Bio Labs, Beverly, Mass.). The ligation mixture was used as a template in a standard polymerase chain reaction (PCR) (26) with a pair of the HIV-1 LTR specific primers: LTR 1 for the U3 region (5'-CCT GAT TAG CAG AAC TAC ACA CCA-3') and AA55 for the R region (68). The PCR conditions were 1 min at 55°C, 1 min at 72°C, and 1 min at 94°C for 40 cycles followed by an extension step of 10 min. The PCR products were resolved on a 1.5% agarose gel, transferred to a Nytran nylon membrane, and hybridized with ³²P-labeled oligonucleotide M669 (68) as a probe under standard conditions (26). In addition to DNA from infected and uninfected cells, three recombinant HIV-1 DNA constructs were used as controls in the procedure. The pN1T-A2 plasmid, which contains the N1T-A provirus from CEM/N1T-A cells, including cellular flanking sequences bounded by *XbaI* restriction sites (47), served as the positive control for integrated HIV-1. The 0.72-kbp *SacI-XhoI* HIV-1 LTR fragment excised from the HIV-1 LTR-carrying pU3RIII plasmid (56) served as a marker for digestion fragments smaller than 1 kbp, and the 8.9-kbp *SacI-SacI* full genomic HIV-1 DNA fragment excised from the pN1T-E2 plasmid (47) served as a marker for a full-length HIV-1 DNA. The markers were spiked into HIV-1-negative cell DNA, and the DNA mixture was analyzed in the HIV-1 DNA integration assay from step I as described above to confirm that the LMP gel separation and elution of DNA removed DNA fragments outside of the desired 1- to 5-kbp range.

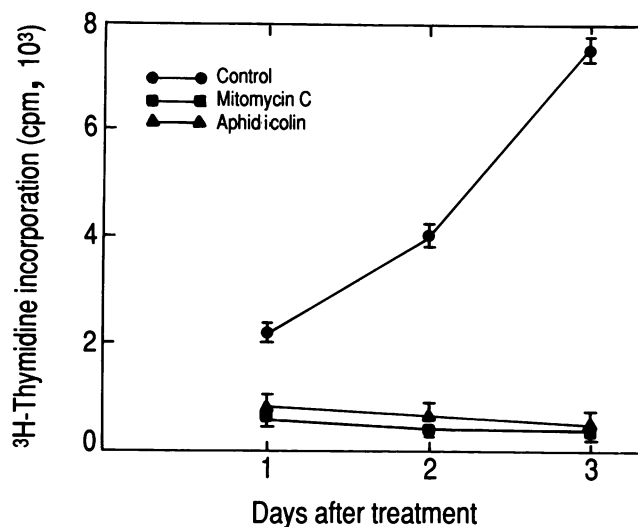


FIG. 1. Arrest of the C8166 cell DNA synthesis by mitomycin or aphidicolin treatment. Cells were treated with mitomycin (0.5 μ g/ml) for 1 h at 37°C or were incubated continuously in the presence of aphidicolin (0.5 μ g/ml), and cellular DNA synthesis was assayed by measuring incorporation of [³H]thymidine, as described in Materials and Methods.

RESULTS

HIV-1 replication and production of infectious virus in nondividing T cells. To resolve the links between HIV-1 replication, cellular activation, and cellular DNA synthesis,

we have established a system for HIV-1 infection of growth-arrested HTLV-1-transformed T cells (C8166). These cells have been shown to rapidly and extensively replicate HIV-1 upon infection (57). Cells were pretreated with mitomycin to cross-link cellular DNA, and thus block new cellular DNA synthesis and cell division (53), and were then infected with HIV-1/HTLV-III_B. Alternatively, cells were infected in the presence of aphidicolin, an inhibitor of cellular DNA polymerase α (67). Assays of [³H]thymidine incorporation into cellular DNA showed that treatment with either of the drugs arrested synthesis of cellular DNA (Fig. 1). That this resulted in the arrest of cell division in drug-treated cells was further confirmed by enumerating living cells by using the assays of tetrazolium salt (MTT) metabolism (40) or trypan blue exclusion (not shown). Under the conditions chosen, about 50% of nondividing cells remained viable 48 h after drug treatment; consequently, 36 to 48 h after infection has been chosen as the end point of the infectivity studies described here.

Figure 2 shows the results of two independent experiments in which we tested the course of HIV-1 infection in growth-arrested C8166 cells. Consistent with other reports (55, 57), HIV-1 at the dose used infected over 90% of C8166 cells as determined by IF within 3 days of virus exposure; 1 to 1.5 μ g of HIV-1 p24 core antigen per ml was detected in culture supernatants at that time (Fig. 2). Viral protein production and the proportion of infected cells were similar in untreated and mitomycin-treated cells. Similar results have been obtained in six experiments. These results suggest that cell division per se is not required for highly productive HIV-1 infection of T cells.

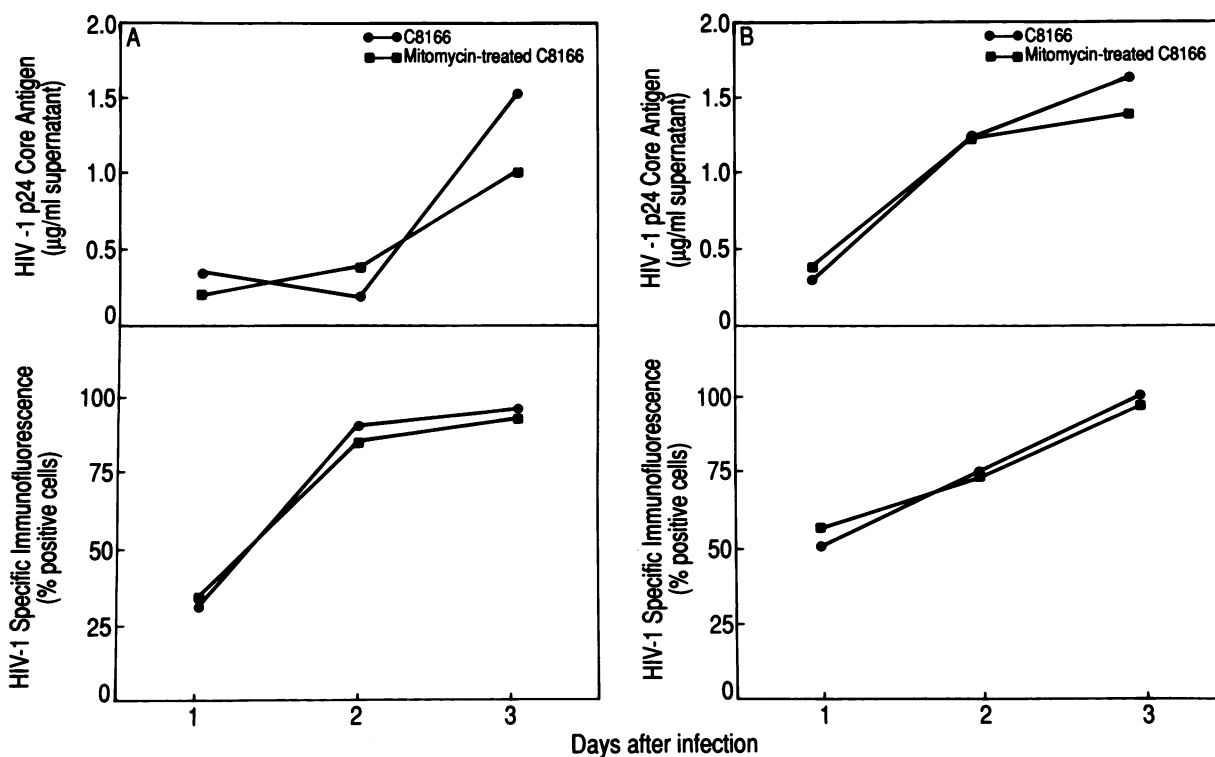


FIG. 2. HIV-1 replication in mitomycin-treated nondividing cells. C8166 cells were pretreated with the drug, infected, and evaluated for HIV-1 expression, as described in Materials and Methods. Panels A and B show the results of two independent experiments.

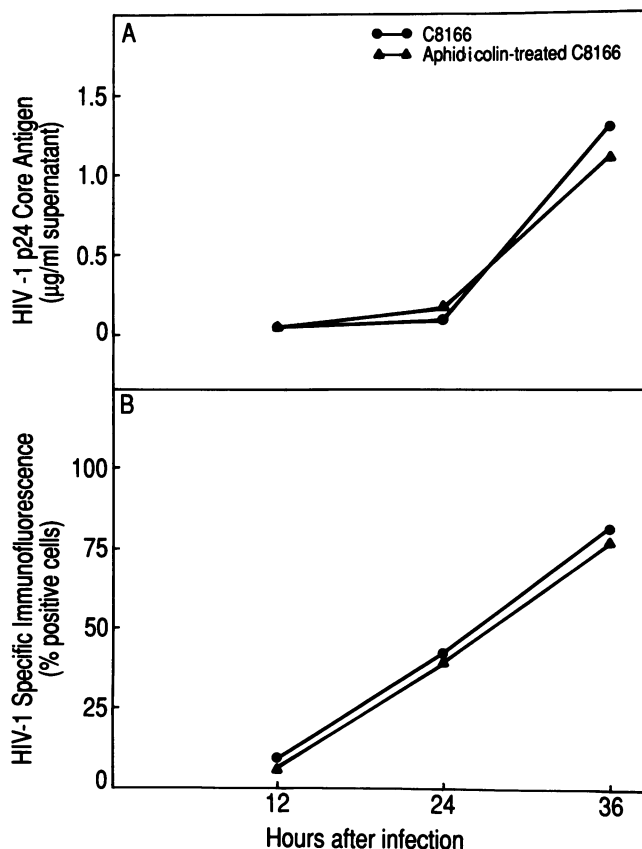


FIG. 3. HIV-1 replication in aphidicolin-treated nondividing T cells. C8166 cells were pretreated with aphidicolin, infected, cultured in the presence of the drug, and evaluated for HIV-1 infection as described previously. Results of a representative experiment of three performed are shown.

Given the mode of action of mitomycin (53), some low-level DNA synthesis undetectable in the [3 H]thymidine assay could continue in the drug-treated cells, contributing to the process of HIV-1 replication. To exclude this possibility, we infected C8166 cells with HIV-1/HTLV-IIIB in the presence of aphidicolin to specifically block the cellular DNA polymerase α (Fig. 3). Viral p24 and IF were monitored. Similar to the results obtained with mitomycin-treated cells, growth arrest of C8166 cells by aphidicolin had little effect on the virus parameters measured. In some experiments, the proportion of IF-positive cells was lower in HIV-1-infected aphidicolin-treated cells than in mitomycin-treated cells (not shown); however, productive infection was observed in either case. Thus, both cell division and cellular DNA synthesis are dispensable for productive HIV-1 infection in T cells.

To determine whether all necessary virus functions were carried out during infection of growth-arrested cells, progeny viruses produced by mitomycin-treated and untreated cells were compared for infectivity (Fig. 4). We used infected cell supernatants taken 3 days after infection from the experiment whose results are shown in Fig. 2 (left panel). The input virus dose (in p24 antigen content) in the supernatants of untreated cells was higher than that in supernatants of mitomycin-treated infected cells (Fig. 2), resulting in higher initial replication of the progeny virus. However, the

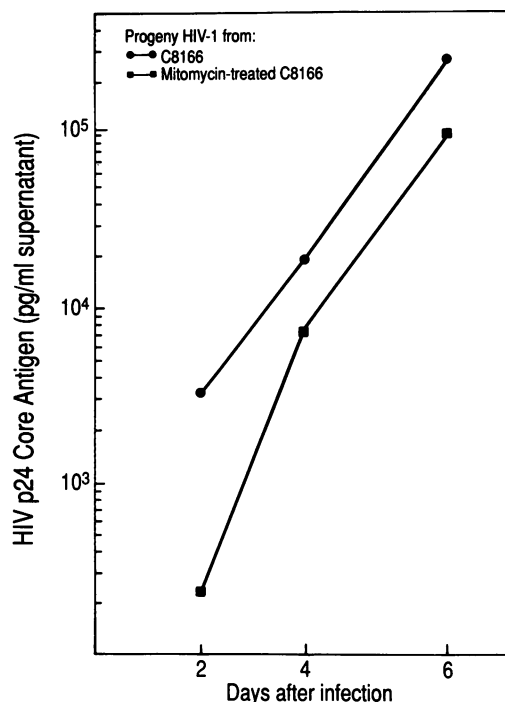


FIG. 4. The biological activity of virus produced in HIV-1-infected nondividing C8166 cells. Day 3 supernatants from the experiment whose results are shown in Fig. 2 (panel A) were used to infect CEM cells (1 ml of supernatant per 10^6 target cells) in a standard protocol. Cells were evaluated for HIV-1 infection as described.

increase in virus titer during the 6-day infection was equivalent to those in progeny of virus replicating in mitomycin-treated or untreated cells. Similar results were obtained when progeny virus cell culture supernatants were sedimented and standardized by their p24 antigen content prior to testing for infectivity (not shown). These findings indicate that the HIV-1 produced in growth-arrested cells is not defective.

To ensure that the results obtained in the experiments described above were not due to some unusual feature of the C8166 cell line or to the specific virus strain used, we evaluated the course of HIV-1 infection in growth-arrested Jurkat cells and employed a different laboratory strain of the virus, HIV-1/N1T (Table 1). Jurkat cells were treated either with mitomycin or aphidicolin and exposed to HIV-1/N1T at a multiplicity of infection of 1, as described above for C8166 cells. Assay of [3 H]thymidine incorporation confirmed that treatment with either drug abolished Jurkat cell DNA synthesis (Table 1). Similar to nondividing C8166 cells, growth-arrested Jurkat cells efficiently replicated HIV-1 (Table 1). The kinetics of HIV-1 infection are slower in Jurkat cells than in C8166 cells (58); hence, the overall levels of virus expression in Jurkat cells 2 days after infection were lower than those in C8166 cells (Table 1). This experiment was repeated three times; similar results were obtained.

HIV-1 DNA synthesis in growth-arrested T cells. To directly evaluate the products of reverse transcription in HIV-1-infected nondividing C8166 cells, cells were treated and infected as shown in Fig. 2 and 3 and were subjected to Hirt DNA extraction for the isolation of extrachromosomal DNA 36 h after infection (Fig. 5). The HIV-1 p24 core

TABLE 1. HIV-1 infection in growth-arrested Jurkat cells^a

Treatment	[³ H]thymidine incorporation into cellular DNA (cpm \pm SD/fold increase) after:			HIV-1 expression 48 h after infection	
	24 h	48 h	72 h	IF (% positive cells)	p24 core antigen (pg/ml of supernatant)
None	3,200 \pm 129 (1)	7,051 \pm 823 (2.2)	13,829 \pm 121 (4.3)	17	6.7 \times 10 ⁴
Mitomycin (0.25 μ g/ml)	958 \pm 10 (1)	858 \pm 30 (0.9)	236 \pm 19 (0.3)	13	4.1 \times 10 ⁴
Aphidicolin (0.25 μ g/ml)	1,401 \pm 127 (1)	951 \pm 44 (0.7)	564 \pm 59 (0.4)	12	3.7 \times 10 ⁴

^a Cells were pretreated with mitomycin or aphidicolin, washed, and tested in parallel for cellular DNA synthesis and susceptibility to HIV-1 infection as described in Materials and Methods. Aphidicolin was present in the culture medium throughout the testing period.

antigen levels at that time were 3.0, 2.4, and 2.8 μ g of p24 per 10⁶ cells in untreated, aphidicolin-treated, and mitomycin-treated cells, respectively. Linear, circular, and high-molecular-weight viral DNA forms were present at similar ratios in all three treatments (Fig. 5). In the majority of our experiments, steady-state levels of HIV-1 DNA in treated and untreated cells, as determined by Southern blot analysis, closely paralleled HIV-1 protein production as measured in p24 and IF assays. Similar results were obtained in drug-treated and untreated HIV-1-infected Jurkat cells (not shown). Thus, under the conditions described here, there appear to be no significant differences in viral DNA synthesis or circularization in aphidicolin- or mitomycin-treated cells and untreated cells. Others have reported recently that accumulation of circular, but not linear, HIV-1 DNA was inhibited in aphidicolin-treated HUT-78 cells infected with HIV-1 (35). We attribute this difference to the relatively high concentration of aphidicolin used in the reported study, 6.7 μ g/ml (35). We found that aphidicolin concentrations above 0.5 μ g/ml were highly cytotoxic in our systems.

Next, we analyzed viral DNA present in the high-molecular-weight DNA fraction of treated or untreated cells by Southern blot hybridization (Fig. 6). The HIV-1 expression at the time of sampling in this experiment was 2.1 and 2.0 μ g of HIV-1 p24 core antigen per 10⁶ mitomycin-treated and untreated cells, respectively. With *Sac*I and *Hind*III restriction endonucleases, the restriction patterns of total viral DNA were found to be similar in mitomycin-treated and untreated cells. It should be noted that the *Sac*I digestion of

virus-infected C8166 cellular DNA produced only two major HIV-1 DNA fragments, of 8.9 and 0.6 kbp (Fig. 6). The two intragenomic fragments of 5.5 and 3.5 kbp described in the original HTLV-III_B producer cell line (41, 54) were absent, indicating that our HTLV-III_B virus preparation contained predominantly the HTLV-III_B quasiespecies lacking the internal *Sac*I restriction site, such as HXB-3 or BH-10 (54). In the absence of digestion, HIV-1 DNA was detected as a set of bands of about 9 kb and above in size; no qualitative or quantitative difference between treated and untreated cells was observed. These results demonstrate that high-molecu-

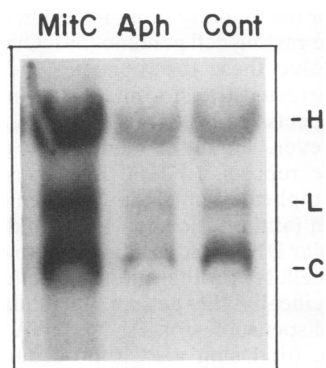


FIG. 5. Comparison of extrachromosomal HIV-1 DNA accumulation in HIV-1 infected nondividing T cells. Hirt DNAs were extracted from C8166 cells 36 h after drug treatment and infection and were subjected to Southern blot analysis with a ³²P-labeled HIV-1-specific probe as described in Materials and Methods. MitC, mitomycin; Aph, aphidicolin; Cont, control; C, L, and H, circular, linear, and high-molecular-weight DNAs, respectively.

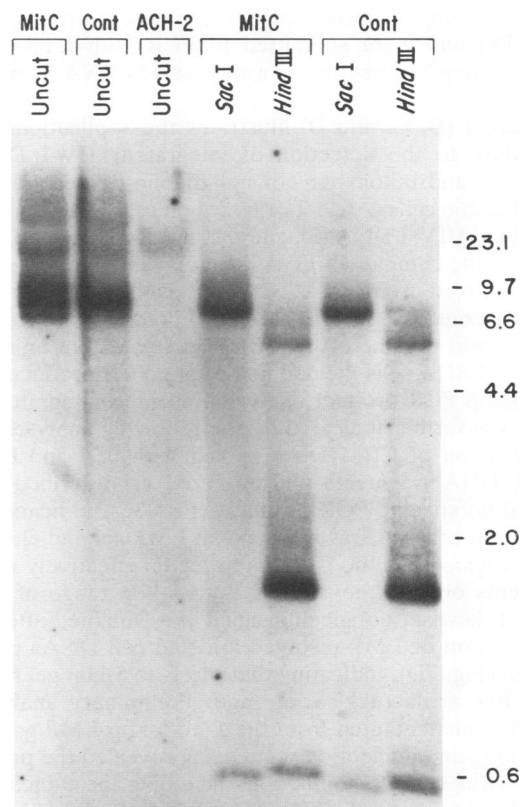


FIG. 6. Restriction endonuclease pattern of HIV-1 DNA present in the high-molecular-weight DNA fraction of growth-arrested T cells. High-molecular-weight DNA was extracted from C8166 cells 36 h after infection of mitomycin (MitC)-pretreated (2 μ g/ml) or untreated (control [Cont]) cells, subjected to digestion with *Sac*I or *Hind*III, and analyzed by Southern blotting and hybridization with ³²P-labeled HIV-1-specific probe as described in Materials and Methods.

lar-weight cellular DNA from antimitotic drug-treated and untreated HIV-1-infected cells contained similar amounts of HIV-1 DNA, which yielded indistinguishable patterns upon endonuclease digestion. Thus, HIV-1 DNA synthesis required for highly productive infection in C8166 T-lymphoid cells takes place independently of cellular DNA synthesis.

HIV-1 DNA integration in growth-arrested T cells. The efficient HIV-1 production (Fig. 2 to 4) and the presence of HIV-1 DNA in unrestricted high-molecular-weight cellular DNA (Fig. 6) suggested that viral DNA in nondividing cells entered the nucleus and was probably integrated into the cellular genome (33, 48). To demonstrate integration of HIV-1 DNA directly, we used a PCR-based procedure (54a) depicted schematically in Fig. 7A. We used viruses and viral clones which yield predictable restriction fragments upon digestion of cellular DNA with *Xba*I and *Sst*I (step I in Fig. 7A). To overcome the problem of the heterogeneous sizes of the HIV-1-cell DNA junction fragments (29, 32), the *Xba*I-*Sst*I restriction fragments were first separated on an LMP gel and extracted in an arbitrarily chosen range of 1 to 5 kbp (steps II and III in Fig. 7A). The 1- to 5-kbp-sized group is not expected to contain unintegrated HIV-1 DNA because, in the infectious viral DNAs used here, *Sst*I digestion of unintegrated DNA (whether linear or circular) produces one 8.9-kbp fragment and a series of small fragments less than 0.7 kbp (47). The isolated DNA fragments were then subjected to ligation to reconstruct a full-size LTR (step IV) and were submitted to PCR with LTR-specific primers (step V), resulting in a single-sized DNA fragment of 561 bp.

Figure 7 (B, C, and D) illustrates the application of this procedure to the detection of integrated HIV-1 DNA in untreated and mitomycin- or aphidicolin-treated HIV-1-infected C8166 cells. ACH-2 cells, which carry 1 to 2 copies of integrated HIV-1 DNA (8), and the pN1T-A2 plasmid, which contains the complete N1T-A provirus (and flanking cellular DNA) cloned from CEM/N1T cell DNA (47), served as positive controls. In all cases, the predicted 561-bp PCR product was obtained (Fig. 7B). The DNA from uninfected CEM or C8166 cells yielded no specific PCR products. That the 561-bp PCR product likely represented integrated viral DNA was further indicated by the following analyses. PCR amplification of CEM DNA spiked with 0.7- and 8.9-kbp HIV-1 DNA fragments and analyzed as described above yielded no specific PCR products (Fig. 7C), indicating that separation of DNA fragments on an LMP gel and elution of DNA fragments in the 1- to 5-kbp region effectively remove fragments outside the desired 1- to 5-kbp range of HIV-1 DNA. Likewise, no specific signal was obtained after PCR amplification of LMP-resolved infected cell DNAs prior to ligation (Fig. 7D), indicating that the 1- to 5-kbp gel fraction has a low artifactual background. Preliminary analysis of DNA fragments eluted from the 1- to 5-kbp LMP gel range by cloning and nucleotide sequencing revealed the presence of the virus-cell DNA junctions including the typical dinucleotide sequences (5'-TG...CA-3') at the boundaries of virus-cell DNA (32) (not shown), confirming that this fraction contains integrated HIV-1 DNA. Taken together, these analyses strongly suggest that HIV-1 DNA integrates during productive infection of mitomycin- or aphidicolin-treated C8166 cells. We conclude that the arrest of C8166 cell division or cellular DNA synthesis does not preclude nuclear entry and HIV-1 DNA integration.

DISCUSSION

The findings presented in this report indicate that cell division and cellular DNA synthesis are dispensable for HIV-1 DNA synthesis, integration of proviral DNA into the cellular genome, and efficient viral replication in T cells. This conclusion is consistent with the early study by McDougal et al., who showed that HIV-1 replicates efficiently in activated but nonproliferating (irradiated) primary T lymphocytes (36). Further support is obtained from a recent study by Li and Burrell, who demonstrated that resting T lymphocytes cocultivated with HIV-1-infected cells synthesize full-length HIV-1 DNA (virus production in these cocultures was not tested) (35). In our model, the established C8166 and Jurkat T-cell lines share some similarities with activated T lymphocytes in that they already express functions required for the completion of the HIV-1 life cycle. The susceptibility of C8166 cells to rapid and highly productive HIV-1 infection (57) permitted analysis of both the HIV-1 DNA forms synthesized and viral DNA integration under the conditions of either continuing or blocked cellular DNA synthesis. We found no difference between the two conditions in the parameters of the viral replicative cycle tested. Other T-cell lines have shown altered virus production and enhanced cell death upon aphidicolin treatment (35, 60). The differences between the systems may lie in the drug concentrations used (up to 0.5 μ g/ml in our study compared with 6.7 μ g/ml in reference 35) and the rapidity of HIV-1 replication in cell populations suffering the toxic effects of the drug. It has been previously established that HIV-1 replicates efficiently in nonproliferating monocytes and macrophages (50, 65), and a recent report by Lewis et al. demonstrated unimpeded HIV-1 infection and viral DNA integration in growth-arrested epithelial HeLa-T4 cells (34). Together, these results suggest that cell activation and/or differentiation, rather than cell division per se, are the prerequisites for productive HIV-1 infection in both primary host cells of the virus: macrophages and T cells. We postulate that viral replication, which is independent of cell division, is a general and fundamental feature of HIV-1 biology, further distinguishing this and other lentiviruses (20, 21, 37, 42, 70) from oncogenic retroviruses.

Previous studies attributed the block to HIV-1 replication in resting T cells to one of three mechanisms: inefficient virus entry (19), synthesis of incomplete labile HIV-1 DNA forms (68, 69), or the inability of a full-length HIV-1 DNA to integrate into the resting cell genome (59). Our work was not intended to resolve these discrepancies, because we employed growth-arrested transformed T-cell lines, which differ in many respects from resting T cells (2, 19, 28). Two comments, however, should be made about our data in the context of these reports. (i) HIV-1 synthesized complete viral DNA and replicated efficiently in T cells treated with either mitomycin (antimitotic agent) or aphidicolin (specific inhibitor of cellular DNA synthesis) (Fig. 2 to 4 and Table 1). Thus, not only cellular proliferation but cellular DNA synthesis (and specifically the action of cellular DNA polymerase α) are dispensable for HIV-1 DNA synthesis and viral replication. (ii) Using a PCR procedure that detects integrated HIV-1 DNA (Fig. 7), we demonstrated that HIV-1 DNA integrates in both growth-arrested and cellular DNA synthesis-inhibited T cells. This indicates that T-cell division per se is not required for entry of viral DNA into the nucleus or for its integration into cellular DNA.

Our results demonstrate that the arrest of T-cell division or of cellular DNA synthesis does not impede the progress of

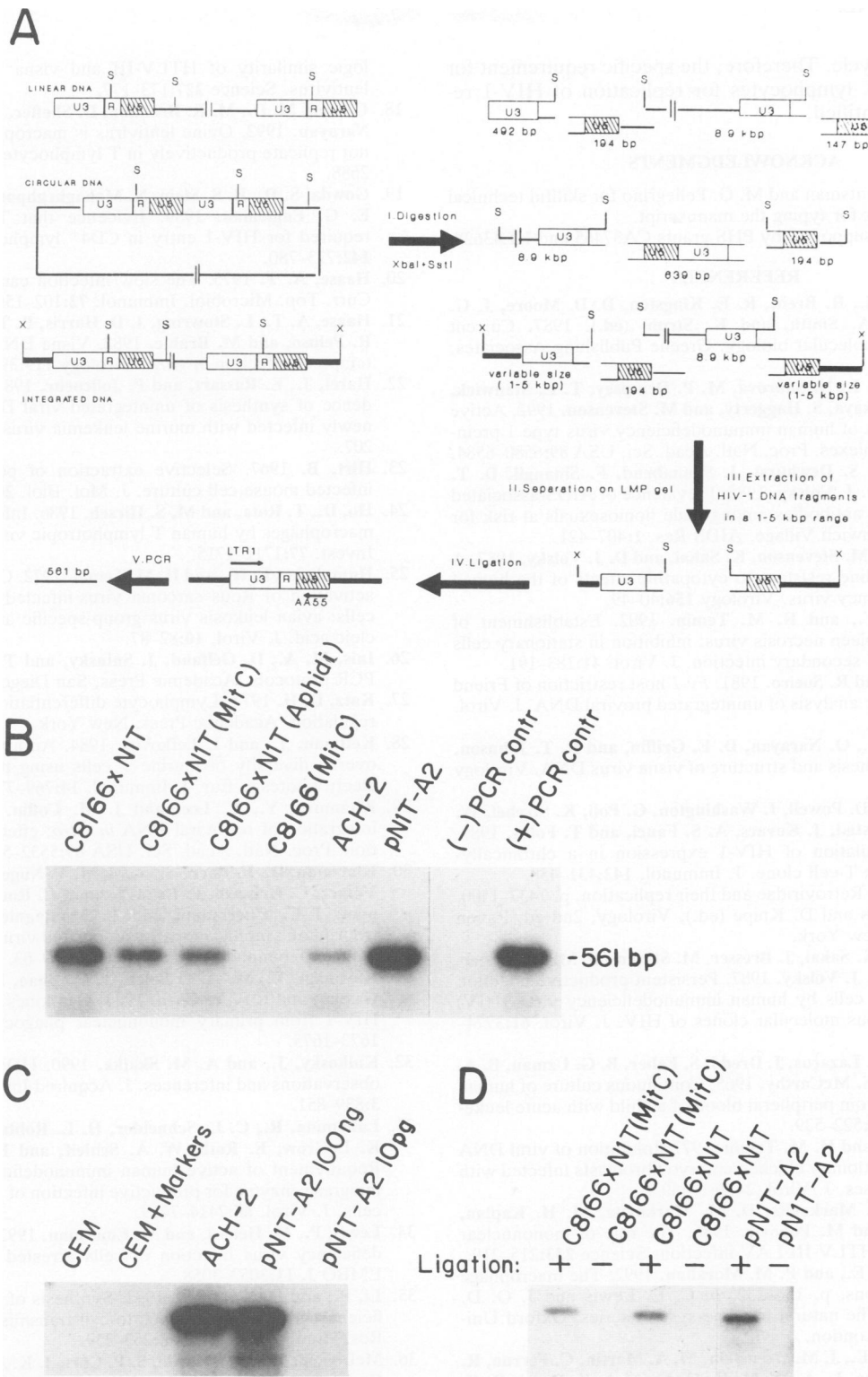


FIG. 7. HIV-1 DNA integration in dividing and growth-arrested T cells. (A) Schematic representation of the PCR method for detection of integrated HIV-1 DNA based on N1T-A (47). See text for details. S, *SstI*; X, *XbaI*. **(B)** Detection of integrated HIV-1 DNA in mitomycin (MitC)- or aphidicolin (Aphidi)-treated C8166 cells. Cells were treated with the drugs and infected as described previously; the HIV-1 expression at the time of analysis (48 h postinfection) was 3, 2.4, and 2.8 μ g of p24 per lysate of 10^6 cells in untreated, aphidicolin-treated, and mitomycin-treated cells, respectively. C8166(MitC), uninfected mitomycin-treated cells; (-) PCR contr and (+) PCR contr, negative and positive PCR controls (Perkin-Elmer), respectively, included in the amplification panel. **(C and D)** Specificity and sensitivity of the HIV-1 DNA integration assay. For details see text. CEM, uninfected CEM cell DNA; CEM + markers, CEM DNA spiked with 0.7- and 8.9-kbp HIV-1 DNA fragments; ACH-2, DNA from ACH-2 cells, which carry one to two copies of integrated HIV-1 DNA (8); pN1T-A2, cloned HIV-1/N1T-A provirus from CEM/N1T cells (47); ligation \pm , analysis of viral DNAs from the systems shown in panel B, including or excluding ligation (step IV).

the HIV-1 life cycle. Therefore, the specific requirement for stimulation of T lymphocytes for replication of HIV-1 remains to be identified.

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